2003. The Journal of Arachnology 31:131–134

SHORT COMMUNICATION

SPIDER PREDATION: SPECIES-SPECIFIC IDENTIFICATION OF GUT CONTENTS BY POLYMERASE CHAIN REACTION

Matthew H. Greenstone[1] and **Kevin A. Shufran**: U.S. Department of Agriculture, Agricultural Research Service, Plant Science and Water Conservation Research Laboratory, 1301 N. Western Street, Stillwater, Oklahoma 74075, USA

ABSTRACT. We extend detection of arthropod predator gut contents by polymerase chain reaction (PCR), heretofore restricted to insect predators, to spiders. Single individuals of the corn lead aphid, *Rhopalosiphum maidis*, were detected in the guts of spiderlings of *Oxyopes salticus* up to 12 h after feeding; individuals of the congeneric bird cherry oat aphid, *R. padi*, were not detected. Unfed *O. salticus* and *Misumenops* sp. were also negative.

Keywords: Polymerase chain reaction, PCR, predation, gut analysis

Spiders tend to be small, cryptic feeders and, having extra-oral digestion and sucking mouthparts, exhibit amorphous gut contents; all of these attributes make it very difficult to obtain data on predation rates (Stuart & Greenstone 1990). Some information can be gathered by direct observation (Greenstone 1999), but gut analysis of field-collected spiders is the least disruptive and most efficient means to acquire data on predation (Stuart & Greenstone 1990).

The state-of-the-art for arthropod predator gut analysis has been serological assay. When monoclonal antibodies are used, specificity can be exquisite, extending to the species, stage, and even instar level (Greenstone & Morgan 1989; Symondson & Liddell 1993; Greenstone & Trowell 1994; Hagler et al. 1994; Ruberson & Greenstone 1998; Agustí et al. 1999a; Symondson et al. 1999; Harwood et al. 2001). Nevertheless, the production of monoclonal antibodies is an expensive and involved process comprising scores of steps with stochastic determinants of success (Greenstone 1996), and although monoclonal antibodies were described more than 25 years ago (Köhler & Milstein 1975), only a handful of arthropod ecologists have used them to study predation.

An appealing alternative is the detection of prey DNA in predator guts (Agustí et al. 1999b, 2000; Zaidi et al. 1999; Chen et al. 2000). The approach has several advantages: (1) the techniques necessary to develop molecular probes are widely known and in some cases have been subsumed into commercial kits; (2) a variety of candidate target regions have already been sequenced in insects, providing information on their variability and hence suitability as probes; (3) once prey species-specific primers have been designed and published, any investigator can have them manufactured cheaply and use them in reproducible protocols.

We have targeted the cytochrome oxidase II (COII) gene in our research on cereal aphid biocontrol. Being a mitochondrial gene, it occurs as multiple copies per cell, which increases the likelihood of successful amplification in gut extracts. It also exhibits various levels of variability (Zhang & Hewitt 1996), allowing closely related species to be separated. Finally, sequences are already available for several aphid species (Rouhbakhsh et al. 1996; Sunnucks & Hales 1996). Here we present the results of a pilot study designed to determine whether a the PCR assay demonstrated to detect cereal aphids species-specifically in insect predators (Chen et al. 2000) will also work in spiders.

Russian wheat aphids, *Diuraphis noxia* (Mordvilko), corn leaf aphids, *Rhopalosiphum maidis* (Fitch), and bird cherry-oat aphids, *R. padi* (L.), from colonies at the USDA-ARS Plant Science Research Laboratory in Stillwater, Oklahoma, were maintained at ≈25°C and a photoperiod of 12:12

[1] Current address: USDA-Agricultural Research Service, Insect Biocontrol Lab, Bldg. 011A, Rm. 214, BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

(light:dark) h on wheat. Second and third instars of the striped lynx, *Oxyopes salticus* Hentz 1845, and spiderlings of an unidentified species of *Misumenops* F.O.P.-Cambridge 1900, were collected by D-vac from wheat and alfalfa fields at the Oklahoma State University North Central Research Station in Chickasha, Oklahoma, on 29 November 2000. Voucher specimens have been deposited in the Cereal Genetics Research Library of the USDA-ARS Plant Science and Water Conservation Research Laboratory.

Spiders were starved for 1 d, placed in an incubator simulating field temperatures at mid-canopy level in a wheat field at the same locality in the spring of 1999 (Chen et al. 2000), and offered 3 *D. noxia*. Experimental spiders, all *O. salticus*, were then starved for an additional 3 d before being offered a single *R. maidis*, offered five additional *D. noxia* to simulate continued feeding, placed back into the incubator, and then killed by freezing at 4 h (four individuals) or 12 h (six individuals) post-feeding; those that did not consume the corn leaf aphid within 1 h were dropped from the experiment. Two control *O. salticus* were fed a single *R. padi* and killed after consuming it. Additional starved spiders, one of each species, were included as a check against false positives due to amplification of spider DNA. The first 4 d of this protocol were designed to ensure that DNA from any *R. maidis* ingested in the field would have been rendered undetectable before the spiders were killed.

We modified the methods of Zhu & Greenstone (1999) to extract total DNA. Insects or spiders were placed individually in 1.5-ml microcentrifuge tubes and homogenized using a battery-powered homogenizer (Midwest Scientific, St. Louis, MO) in 100 µl or 500 µl, for aphids and spiders, respectively, of isolation buffer (Chen et al. 2000). The homogenate was vortexed briefly and incubated for 30 min at 65°C. The solution was transferred to a new tube and extracted once with one volume of chloroform/isoamyl alcohol (24:1). One-tenth volume of 3.0 M sodium acetate and two volumes of ice-cold 100% EtOH were added to the tube. DNA was then pelleted by centrifugation, dried, and resuspended in 200 µl distilled water.

Protocols for the design of species-specific mitochondrial COII primers for six cereal aphid species have been given elsewhere (Chen et al. 2000). PCR reactions, using *R. maidis* primers ClaCOIIF and ClaCOIIR1 (Table 2 of Chen et al. 2000), were performed as described by Chen et al. (2000). PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and photographed under UV light.

All *O. salticus* fed *R. maidis* were positive by PCR for 4 h and 12 h post-feeding. All unfed *O. salticus* and *Misumenops* sp., and *O. salticus* fed *R. padi*, were negative (Fig. 1). These negatives demonstrate either that the first 4 d of the experimental protocol were sufficient to render all *R. maidis* DNA in the guts of the experimental *O. salticus* undetectable, or that the animals had not consumed *R. maidis* in the field prior to capture.

Figure 1.—PCR amplification of *Oxyopes salticus* fed *Rhopalosiphum maidis*. Lanes 1, 10 and 20: 100 bp DNA ladder. Lane 2: *R. padi* DNA. Lane 3: *R. maidis* DNA. Lanes 4–7: *O. salticus* fed *R. maidis* killed 4 h after feeding. Lanes 8–9 and 11–14: *O. salticus* fed *R. maidis* killed 12 h after feeding. Lane 15: Unfed *O. salticus*. Lane 16: Unfed *Misumenops* sp. Lanes 17 and 18: *O. salticus* fed *R. padi*. Lane 19: Negative control (no template).

This is the first report of spider gut analysis by PCR. By focusing on two aphid congeners, we have made a very stringent case for specificity. The assay will detect 10^{-7} aphid equivalent (Chen et al. 2000). If run in a microplate format, an individual PCR assay costs ≈$0.28; this compares favorably to the only technology with similar specificity and sensitivity, ELISA with monoclonal antibodies, at $0.21 (Chen et al. 2000).

Before assay data from field-collected animals can be used, the detectability half-life (Greenstone & Hunt 1993) for a single aphid must be determined. Detectability half-lives are necessary because mere determination of the proportion of predator individuals positive for prey DNA is not a reliable indicator of the relative importance of any given predator taxon. For example, the green lacewing *Chrysoperla plorabunda* has a half-life (3.95 h) for detectability of *R. maidis* DNA that is only 0.45 that (8.78 h) of the ladybird beetle *Hippodamia convergens* (Chen et al. 2000). Consequently, the consumption of a single *R. maidis* is 2.2 times as likely to be detected in an *H. convergens* individual as in a *C. plorabunda* individual; another way to look at it is that a positive *C. plorabunda* is "worth" 2.2 times as much as an *H. convergens* positive.

We may expect to find dramatic differences in detectability half-lives as more predator taxa are studied. For example, in the analogous case of detecting protein antigens in serological predator gut analysis, staphylinid beetles appear to have short detectability half-lives (Sunderland et al. 1987), and spiders much longer ones (Greenstone 1983; Ragsdale et al. 1981; Harwood et al. 2001). Whether such differences between spiders and other arthopod predators will also be found with respect to

DNA digestion will not be known until rigorous comparative studies employing very large sample sizes (cf. Chen et al. 2000) have been conducted.

ACKNOWLEDGMENTS

We thank Yi "Charlie" Chen and Brian Jones for technical support, Raymond Sidwell for assistance in the field, and Jason Bond and James Hardwood for comments on the manuscript.

LITERATURE CITED

Agustí, N., J. Aramburu, & R. Gabarra. 1999a. Immunological detection of *Helicoverpa armigera* (Lepidoptera: Noctuidae) ingested by heteropteran predators: time-related decay and effect of meal size. Annals of the Entomological Society of America 92:56–62.

Agustí, N., M.C. de Vicente, & R. Gabarra. 1999b. Development of SCAR markers of *Helicoverpa armigera*: a new PCR-based technique for predator gut analysis. Molecular Ecology 8:1467–1474.

Agustí, N., M.C. de Vicente, & R. Gabarra. 2000. Developing SCAR markers to study predation on *Trialeurodes vaporareorum*. Insect Molecular Biology 9:263–268.

Chen, Y., K.L. Giles, M.E. Payton, & M.H. Greenstone. 2000. Identifying key cereal aphid predators by molecular gut analysis. Molecular Ecology 9:1887–1898.

Greenstone, M.H. 1983. Site-specificity and site tenacity in a wolf spider: a serological dietary analysis. Oecologia 56:79–83.

Greenstone, M.H. (1996). Serological analysis of arthropod predation: past, present and future. In The Ecology of Agricultural Pests—Biochemical Approaches. W.O.C. (eds. Symondson WOC, Liddell JE), pp. 265–300. Chapman and Hall, London.

Greenstone, M.H. 1999. Spider predation: how and why we study it. Journal of Arachnology 27: 333–342.

Greenstone, M.H. & J.H. Hunt. 1993. Determination of prey antigen half-life in *Polistes metricus* using a monoclonal antibody-based immunodot assay. Entomologia Experimentalis et Applicata 68:1–7.

Greenstone, M.H. & C.E. Morgan. 1989. Predation on *Heliothis zea* (Lepidoptera: Noctuidae): an instar-specific ELISA for stomach analysis. Annals of the Entomological Society of America 82:45–49.

Greenstone, M.H. & S.C. Trowell. 1994. Arthropod predation: A simplified immunodot format for predator gut analysis. Annals of the Entomological Society of America 87:214–217.

Hagler, J.R., S.E. Naranjo, D. Bradley-Dunlop, F.J. Enriquez, & T.J. Henneberry. 1994. A monoclonal antibody to pink bollworm (Lepidoptera: Gelechiidae) egg antigen: a tool for predator gut analysis. Annals of the Entomological Society of America 87:85.

Harwood, J.D., S.W. Phillips, K.D. Sunderland, & W.O.C. Symondson. 2001. Secondary predation: quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. Molecular Ecology 10:2049–2057.

Köhler, G. & C. Milstein. 1975. Continuous culture of fused cells secreting antibody of predefined specificity. Nature 256:495–497.

Ragsdale, D.W., A.D. Larson, & L.D. Newsome. 1981. Quantitative assessment of the predators of *Nezara viridula* eggs and nymphs within a soybean agroecosystem using an ELISA. Environmental Entomology 10:402–405.

Rouhbakhsh, D., C.-Y. Lai, C.D. von Dohlen, M.A. Clark, L. Baumann, P. Baumann. 1996. The tryptophan biosynthetic pathway of aphid endosymbionts (*Buchnera*) genetics and evolution of plasmid-associated anthranilate synthase (trpEG) within the Aphididae. Journal of Molecular Evolution 42:4–421.

Ruberson, J.R. & M.H. Greenstone. 1998. Predators of budworm/bollworm eggs in cotton: an immunological study. Proceedings of the 1998 Beltwide Cotton Production and Research Conference 2:1095–1098.

Stuart, M.K. & M.H. Greenstone. 1990. Beyond ELISA: a rapid, sensitive, specific immunodot assay for identification of predator stomach contents. Annals of the Entomological Society of America 83:1101–1107.

Sunderland, K.D., N.E. Crook, D.L. Stacey, & B.J. Fuller. 1987. A study of feeding by polyphagous predators on cereal aphids using ELISA and gut dissection. Journal of Applied Ecology 24:907–933.

Sunnucks, P. & D.F. Hales. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II aphids of the genus *Sitobion* (Hemiptera: Aphididae). Molecular Biology and Evolution 13:510–524.

Symondson, W.O.C. & J.E. Liddell. 1993. A monoclonal antibody for the detection of arionid slug remains in carabid predators. Biological Control 3:207–214.

Symondson, W.O.C., T. Gasull, & J.E. Liddell. 1999. Rapid identification of adult whiteflies in plant consignments using monoclonal antibodies. Annals of Applied Biology 134:271–276.

Zaidi, R.H., Z. Jaal, N.J. Hawkes, J. Hemingway, & W.O.C. Symondson. 1999. Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? Molecular Ecology 8:2081–2088.

Zhang, D.-X. & G.M. Hewitt. 1996. Nuclear integrations: challenges for mitochondrial DNA

markers. Trends in Ecology and Evolution 11: 247–251.

Zhu, Y.-C. & M.H. Greenstone. 1999. Polymerase chain reaction techniques for distinguishing three species and two strains of *Aphelinus* (Hymenoptera: Aphelinidae) from *Diuraphis noxia* and *Schizaphis graminum* (Homoptera: Aphididae). Annals of the Entomological Society of America 92:71–79.

Manuscript received 13 September 2001, revised 15 July 2002.